

# EXHIBIT 1

(12) **United States Patent**  
**Müller et al.**

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(45) **Date of Patent: Aug. 27, 2002**

(54) **STABLE CRYSTALLINE SALTS OF 5-METHYLTETRAHYDROFOLIC ACID**

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(\*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 0 days.

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(22) Filed: **Apr. 17, 2000**

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Apr. 15, 1999	(CH)	695/99
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(51) **Int. Cl.<sup>7</sup>** ..... **C07D 475/04**

(52) **U.S. Cl.** ..... **544/258**

(58) **Field of Search** ..... **544/258**

(56) **References Cited**

U.S. PATENT DOCUMENTS			
5,006,655	A *	4/1991	Muller et al. .... 544/258
5,194,611	A *	3/1993	Marazza et al. .... 544/258
5,223,500	A *	6/1993	Gennari ..... 514/249
5,300,505	A	4/1994	Muller ..... 514/250
5,324,836	A	6/1994	Muller ..... 544/258
5,332,815	A	7/1994	Molera ..... 544/258
5,350,850	A *	9/1994	Vecchi ..... 544/258
5,382,581	A	1/1995	Marezze ..... 514/245
5,457,202	A *	10/1995	Scheib ..... 544/258
5,817,659	A	10/1998	Muller ..... 514/245

**FOREIGN PATENT DOCUMENTS**

DE	2807393	9/1978
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EP	0455013	11/1991
EP	0495204	7/1992
EP	0535710	4/1993
EP	0537492	4/1993
EP	0539987	5/1993
EP	0682026	11/1995
EP	0773221	5/1997

**OTHER PUBLICATIONS**

Fitzhugh Pteridines 4, 187 (1993).\*

Pauling, Linus, “General Chemistry, 2nd Ed.”, 1953, Freeman, San Francisco, p 22 & 41.\*

Hawley, Gessner, “The Condensed Chemical Dictionary”, 1977, Van Nostrand, New York, p. 240.\*

Loewenthal, H.J.E. “A Guide for the Perplexed Organic Experimentalist, 2nd Ed”., John Wiley & sons, Chichester, 1992, p 145.\*

Wiberg, K.B., “Laboratory Technique in Organic Chemistry”, 1960, McGraw-Hill, New York, p 99.\*

Derwent English abstract of EP 539 987.

Derwent English abstract of EP 682 026.

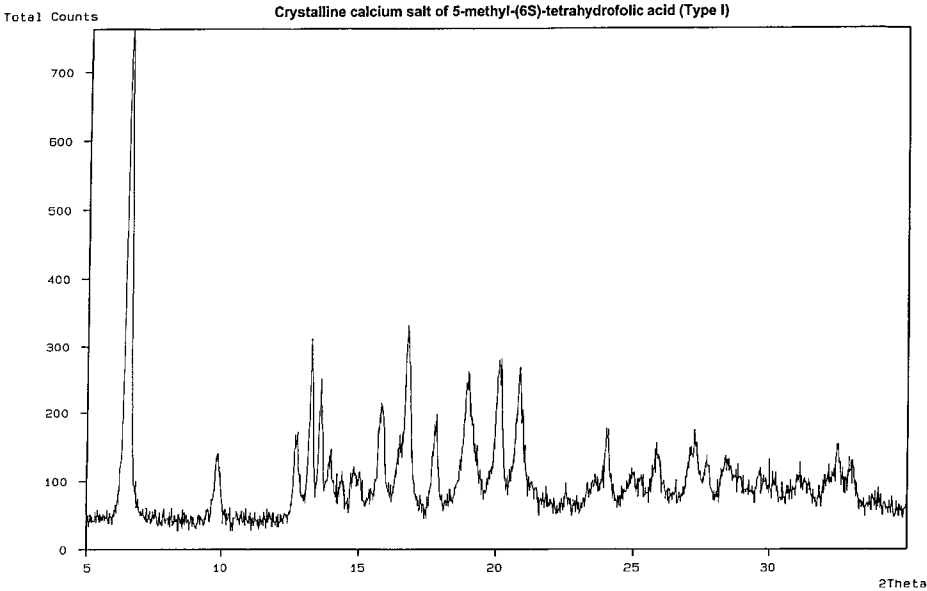
\* cited by examiner

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(57) **ABSTRACT**

This invention relates to stable crystalline salts of 5-methyl-(6R,S)-, -(6S)- and -(6R)-tetrahydrofolic acid, to methods of producing these salts and to the use thereof use as a constituent for the production of drugs or as a food additive, and to preparations containing these salts.

**17 Claims, 5 Drawing Sheets**



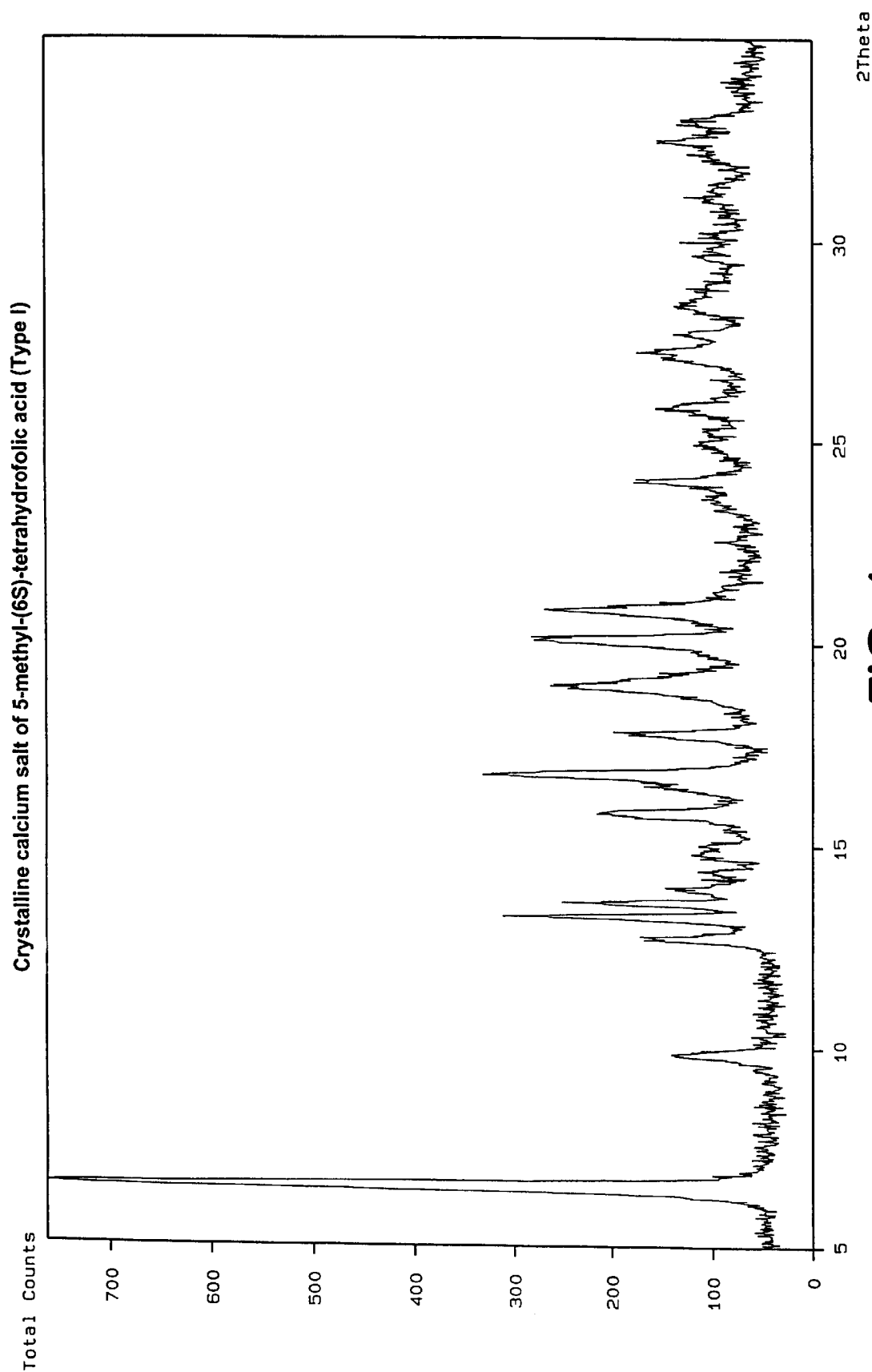


FIG. 1

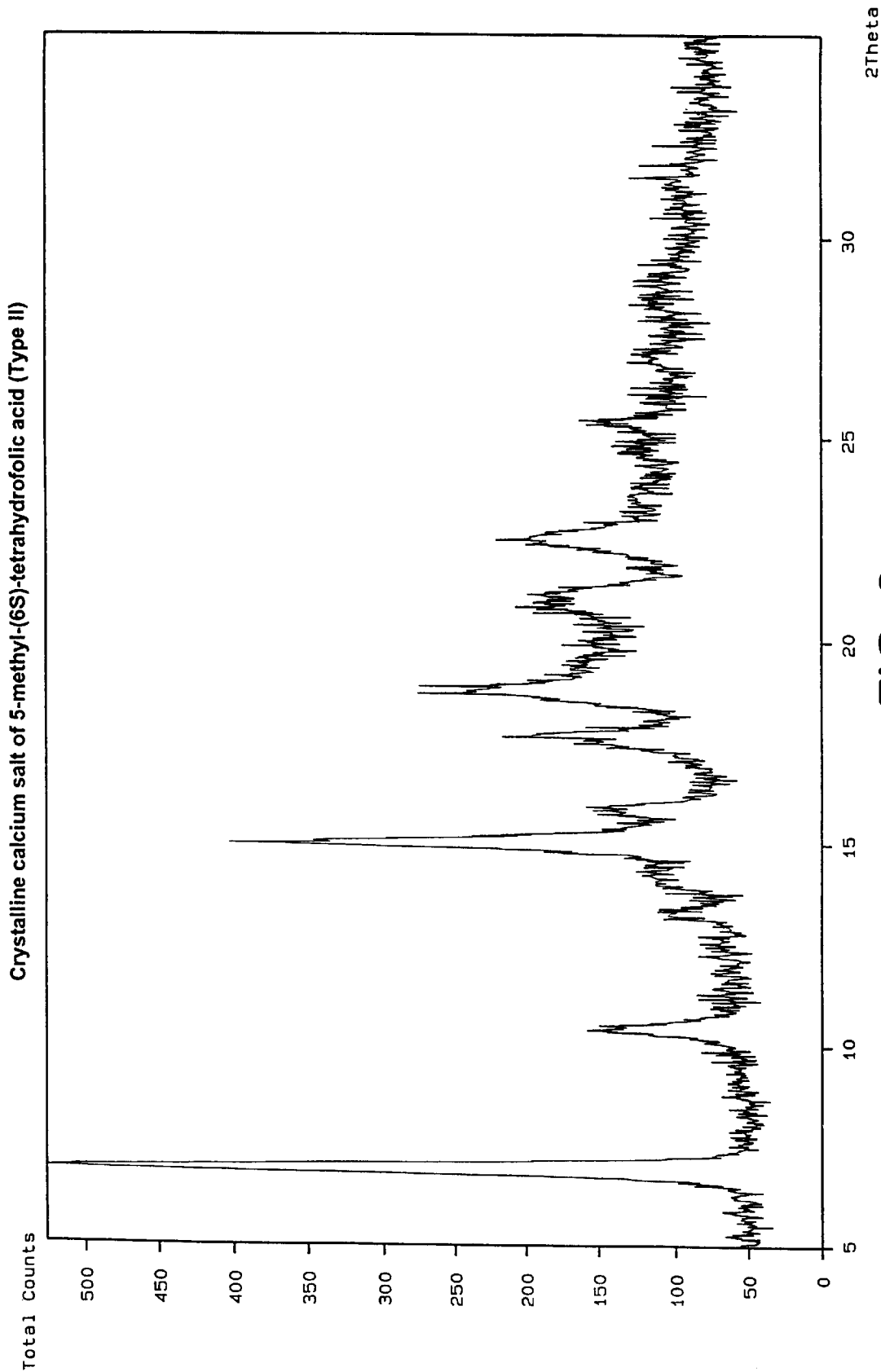


FIG. 2

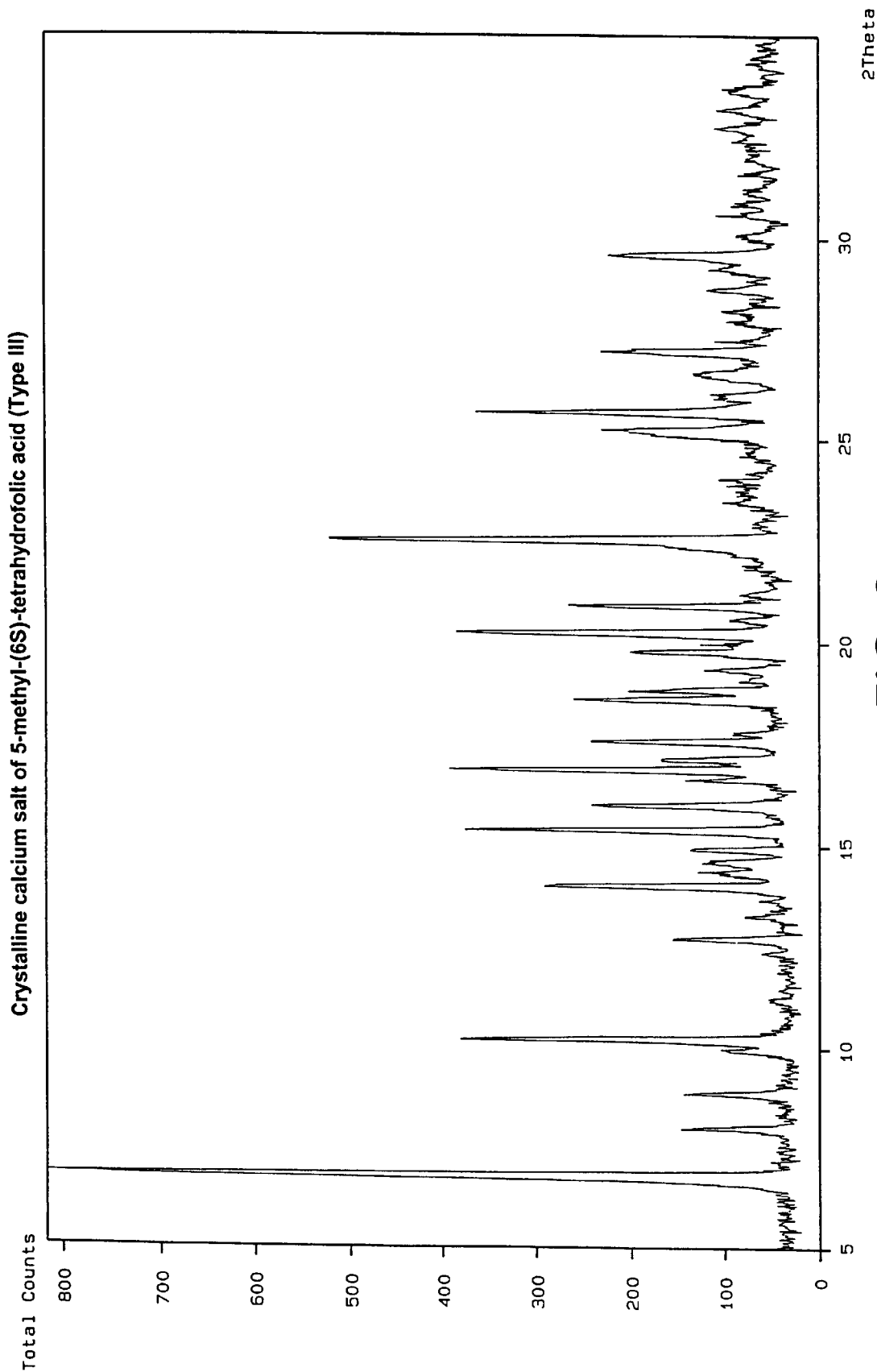


FIG. 3

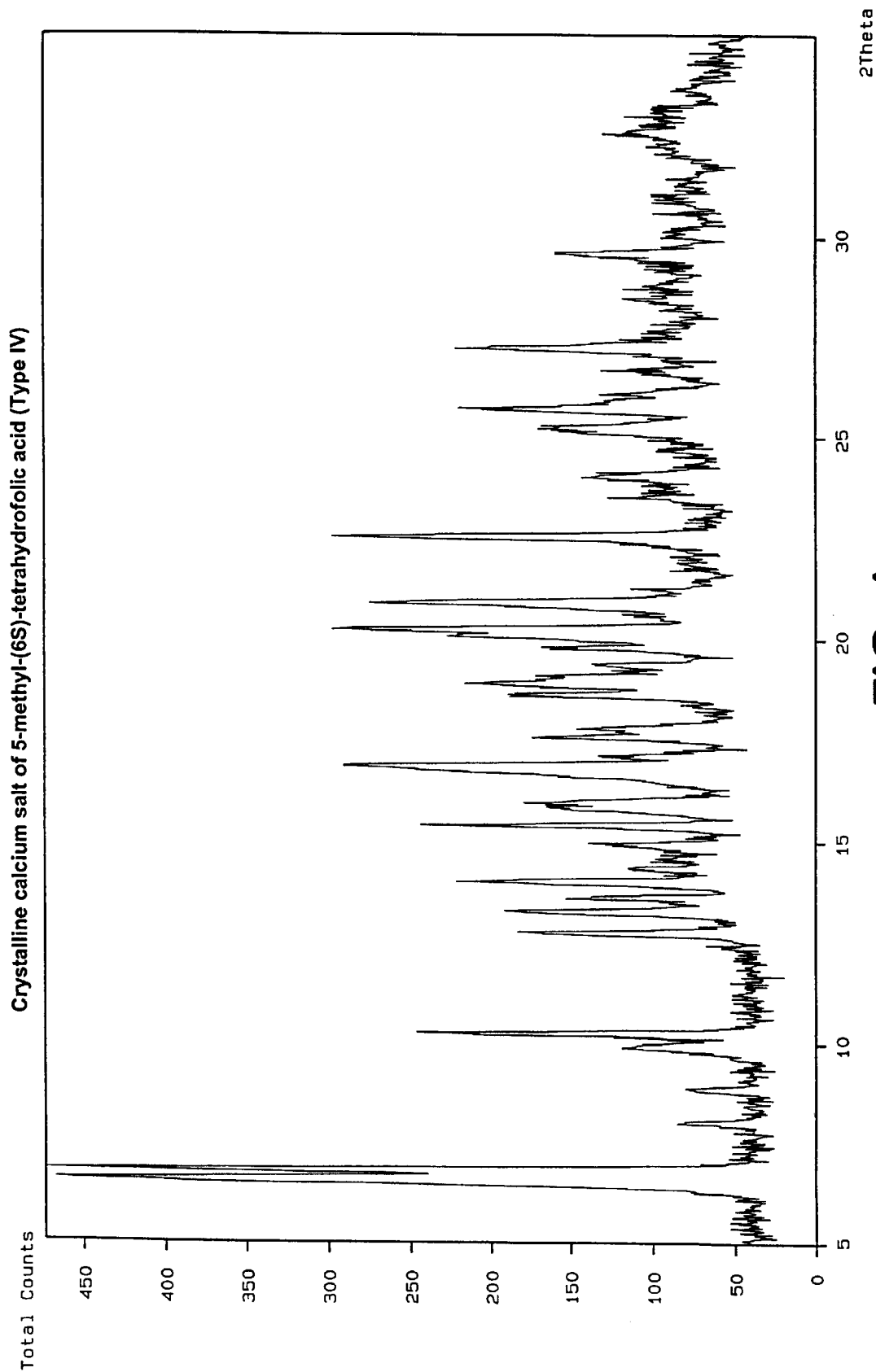


FIG. 4

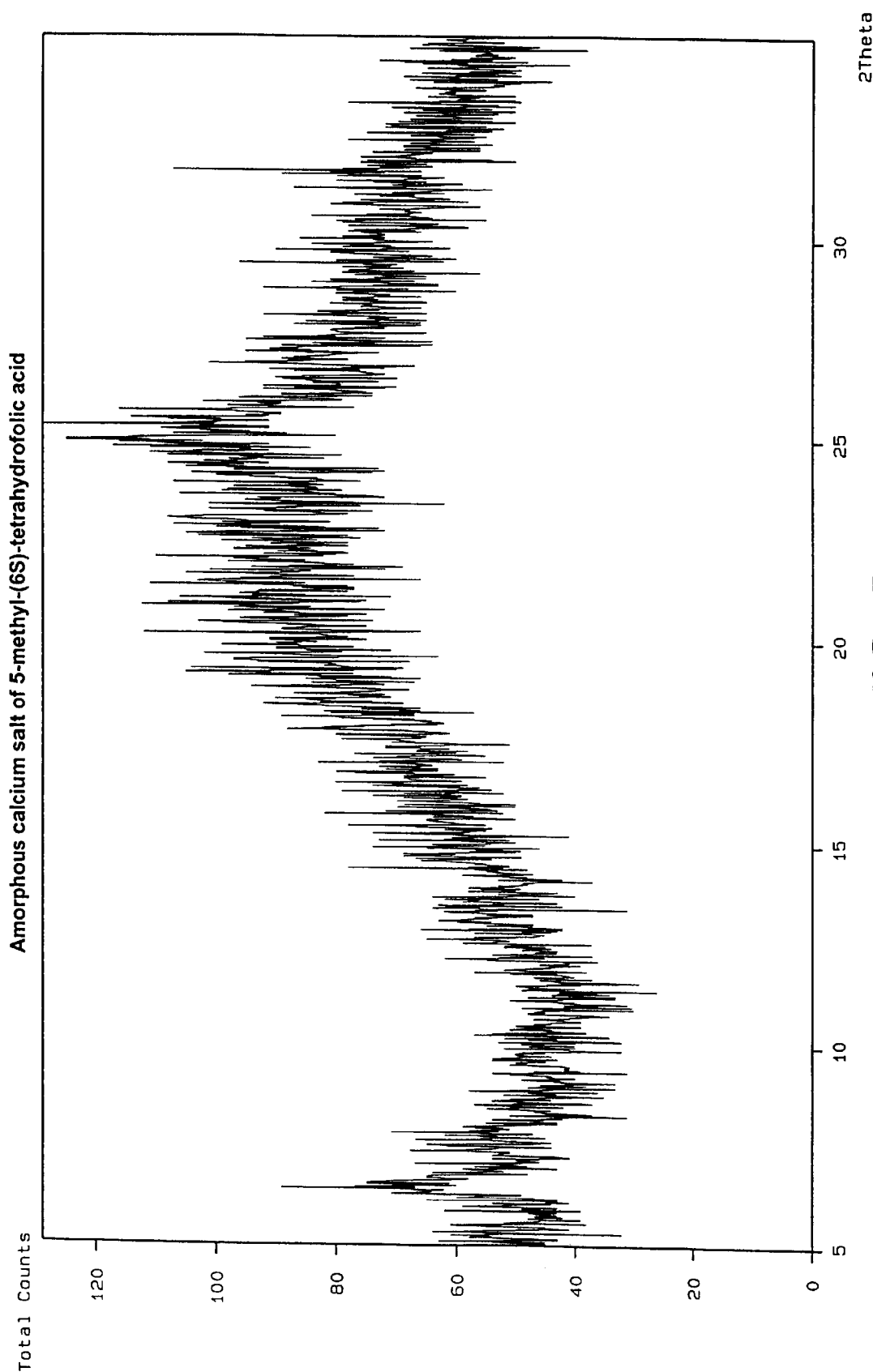


FIG. 5

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**STABLE CRYSTALLINE SALTS OF 5-METHYLTETRAHYDROFOLIC ACID****FIELD OF THE INVENTION**

This invention relates to crystalline salts of N-[4-[(2-amino-1,4,5,6,7,8hexahydro-4-oxo-5-methyl-(6S)-, -(6R)- and -(6R,S)-pteridinyl)methyl]amino]benzoyl-L-glutamic acid (hereinafter called salts of 5-methyltetrahydrofolic acid), to the use thereof, and to a method of producing them.

**BACKGROUND OF THE INVENTION**

Tetrahydrofolates are predominantly used as 5-formyltetrahydrofolic acid and the salts thereof (leucovorin) or as 5-methyltetrahydrofolic acid and the salts thereof, for the treatment of megaloblastic folic acid anaemia, as an antidote for increasing the compatibility of folic acid antagonists, particularly of aminopterin and methotrexate in cancer therapy ("antifolate rescue"), for increasing the therapeutic effect of fluorinated pyrimidines and for the treatment of autoimmune diseases such as psoriasis and rheumatoid arthritis, for increasing the compatibility of certain antiparasitic formulations, for instance trimethoprim-sulfamethoxazole, and for reducing the toxicity of dideazatetrahydrofolates in chemotherapy. 5-methyltetrahydrofolic acid is used in particular as a drug and as a food additive, as a vitamin preparation, for the prevention of neural tube defects, for the treatment of depressive illnesses, and for influencing the homocysteine level.

5-methyltetrahydrofolic acid and salts thereof are extremely unstable, and in particular are highly susceptible to oxidation [see also A. L. Fitzhugh, Pteridines 4 (4), 187-191 (1993) in this respect] and are therefore difficult to produce at a level of purity which is acceptable for a pharmaceutical active ingredient or a food additive.

Various methods, such as excluding oxygen as completely as possible or the addition of antioxidants such as ascorbic acid or reduced L-glutathione, have been employed in order to overcome the instability of 5-methyltetrahydrofolic acid. However, it is scarcely possible completely to exclude oxygen during use, and even then this is only possible at very considerable cost, and the addition of antioxidants is likewise not always possible. Accordingly, it has not been possible hitherto to identify a commercially feasible method which is suitable for the production of salts of 5-methyltetrahydrofolic acid which are satisfactorily stable and which are of high purity.

**SUMMARY OF THE INVENTION**

Surprisingly, it has now been found that salts of 5-methyltetrahydrofolic acid which exhibit high chemical purity and excellent stability can be obtained by crystallising the corresponding salt from a polar medium after subjecting the solution to thermal treatment at a temperature above 60° C. The highly crystalline salts of 5-methyl-tetrahydrofolic acid which are thus obtained are stable at room temperature, practically without-limitation. They are suitable as a constituent or as a starting material for the production of drug forms or food additives.

Accordingly, the present invention relates to crystalline salts of 5-methyltetrahydrofolic acid. Alkaline earth salts, particularly the calcium salt, are preferably used as the salts of 5-methyltetrahydrofolic acid for crystallisation. These crystalline salts of 5-methyltetrahydrofolic acid exhibit a purity, which has never been achieved hitherto, of >98%,

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together with a stability, with respect to the initial value thereof and which has never been achieved hitherto, of >98% after storage for 6 months in air at 25° C. and 60% relative atmospheric humidity. The crystalline calcium salts of 5-methyl-(6S)-tetrahydrofolic acid exist in four different crystalline modifications (Type I, Type II, Type III and Type IV) and exhibit sharp bands when subjected to X-ray powder diffraction measurements (see Table 1 to Table 4 in this respect). Selected 2 theta values for the different crystalline modifications are 6.5, 13.3, 16.8 and 20.1 (Type I); 5.3, 6.9, 18.7 and 21.1 (Type II); 6.8, 10.2, 15.4 and 22.5 (Type III); and 6.6, 15.9, 20.2 and 22.5 (Type IV). Crystalline calcium salts of 5-methyltetrahydrofolic acid have a content of water of crystallisation of at least 1 equivalent of water per 1 equivalent of 5-methyltetrahydrofolic acid. Thus the Type I modification typically contains  $\geq 3$  equivalents of water, the Type II modification typically contains  $\leq 2$  equivalents water and the Type III and Type IV modifications typically contain  $\leq 5$  equivalents of water.

Other Salts of 5-methyl-(6R)-tetrahydrofolic acid and salts of 5-methyl-(6R,S)-tetrahydrofolic acid can likewise be obtained in highly crystalline form.

**BRIEF DESCRIPTION OF THE DRAWINGS**

FIGS. 1-4 are self-explanatory X-ray powder diffraction diagrams of the 4 crystalline modifications of the calcium salts of the invention, and FIG. 5 is a diagram of the amorphous salt.

The present invention further relates to a method of producing highly crystalline salts of 5-methyltetrahydrofolic acid, which is characterised in that the corresponding salt of 5-methyltetrahydrofolic acid is crystallised. In this method, crystallisation of salts of 5-methyltetrahydrofolic acid is effected from a polar medium after thermal treatment at a temperature above 60° C., particularly above 85° C.

Substances which are particularly suitable as the polar medium include water or a mixture of water and an organic solvent which is miscible with water, such as watersoluble alcohols, e.g. methanol, ethanol, n-propanol, iso-propanol or ethylene glycol, a low molecular weight aliphatic water-soluble carboxylic acid e.g. formic acid, acetic acid or lactic acid, or water-soluble amides e.g. formamide, dimethylformamide, dimethylacetamide, 1-methylpyrrolidone, 2-methylpyrrolidone or 2-piperidinone. There are no particular restrictions with regard to the type of solvent used and with regard to the mixture ratio, since crystalline salts of 5-methyltetrahydrofolic acid generally exhibit solubilities which are lower than those of the corresponding amorphous forms.

Crystallisation is preferably effected from solutions. It is also possible to effect crystallisation from a suspension, however.

Aside from calcium salts, still further salts of 5-methyl-(6R,S) or (6R) or (6S) hydrofolic acids include but are not limited to other alkaline earth salts, for example, magnesium can be obtained in highly crystalline forms.

The different crystalline modifications can be converted into one another by further thermal treatments at temperatures above 60° C. Thus Type I, which is produced by crystallisation from a polar medium after thermal treatment at a temperature above 60° C., can be converted into Type II by drying sufficiently, e.g. under vacuum at 70° C., can be converted into Type III by sufficient thermal treatment at a temperature above 90° C., and can be converted into Type IV by sufficient thermal treatment at a temperature above 95° C.



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Type II can be converted into Type 1 again by adding water to the crystals, e.g. by treatment with water in a humidity cabinet at 90° C.

Crystallisation of the salts of 5-methyltetrahydrofolic acid occurs spontaneously or is effected by seeding with the corresponding crystalline salt of 5-methyltetrahydrofolic acid.

Asuitable, preferred starting material for crystallisation is pure, amorphous or crystalline 5-methyl(6S)- or -(6R)-tetrahydrofolic acid. Racemic 5-methyl-(6R,S) tetrahydrofolic acid can also be used, however, as can enriched 5-methyl-(6S)-, -(6R)- or -(6R,S)-tetrahydrofolic acid.

By using amorphous or partly crystalline, optically pure 5-methyltetrahydrofolic acid or salts thereof as the starting material for crystallisation, essentially crystalline salts of 5-methyltetrahydrofolic acid of a purity which has never been achieved hitherto, together with a stability which has never been achieved hitherto, are obtained by the method described here.

The present invention also relates to the use of highly crystalline salts of 5-methyltetrahydrofolic acid as a constituent for the production of drugs or food additive substances or for the production of other tetrahydrofolic acid derivatives, since, on account of their excellent stability in solid form, crystalline salts of 5-methyltetrahydrofolic acid are of a very good quality which remains constant with time, practically without limits. The present invention also relates to preparations containing highly crystalline salts of 5-methyltetrahydrofolic acid. These preparations are produced by known methods. They are employed analogously to the use of known substances from the field of tetrahydrofolates, such as 5-formyltetrahydrofolic acid (leucovorin) for example.

Without further elaboration, it is believed that one skilled in the art can, using the preceding description, utilize the present invention to its fullest extent. The following preferred specific embodiments are, therefore, to be construed as merely illustrative, and not limitative of the remainder of the disclosure in any way whatsoever.

In the foregoing and in the following examples, all temperatures are set forth uncorrected in degrees Celsius and unless otherwise indicated, all parts and percentages are by weight.

EXAMPLES WHICH ILLUSTRATE THE INVENTION

The content of 5-methyltetrahydrofolic acid salt which is quoted in the examples was s determined by HPLC in each case and is given as % area. The water content was determined by a Karl Fischer method.

Example 1  
Stabilities

In order to determine the stabilities of the crystalline salts of 5-methyltetrahydrofolic acid, the substances were stored, together with comparison specimens, in air at 25° C. and at 60% relative humidity. The content of 5-methyltetrahydrofolic acid salt remaining was measured at periodic intervals and is given by comparison with the initial value.

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	Time of storage in months					
	0	3	6	12	18	88
Crystalline calcium salt of 5-methyl-(6S)-tetrahydrofolic acid	100%	98.6%	98.7%	99.1%	99.0%	97.8%
Amorphous calcium salt of 5-methyl-(6S)-tetrahydrofolic acid	100%			84.2%		

The crystalline salts of 5-methyltetrahydrofolic acid were still very light in colour even after an extended period of storage. In contrast thereto, the amorphous samples exhibited considerable discoloration, which occurred very rapidly.

Example 2

X-ray Powder Plots

X-ray powder plots (diffraction spectra) of these substances were recorded in order to characterise the structural properties (crystalline modifications) of the crystalline salts of 5-methyltetrahydrofolic acid.

The crystalline salts of 5-methyltetrahydrofolic acid exhibited spectra of good resolution, with sharp bands and low background effects. The spectra indicated highly crystalline constituents.

Examples of spectra are illustrated in FIG. 1 (Type I), FIG. 2 (Type II), FIG. 3 (Type III) and FIG. 4 (Type IV), and are presented in Table 1 (Type I), Table 2 (Type II), Table 3 (Type III) and Table 4 (Type IV). For comparison, a spectrum of an amorphous sample was also recorded under analogous conditions and is presented as FIG. 5 (amorphous).

Selected 2 theta values for the different crystalline modifications of the crystalline calcium salt of 5-methyl-(6S)-tetrahydrofolic acid are listed below:

Type	Selected 2 theta values
Type I	6.5, 13.3, 16.8 and 20.1
Type II	5.3, 6.9, 18.7 and 21.1
Type III	6.8, 10.2, 15.4 and 22.5
Type IV	6.6, 15.9, 20.2 and 22.5

Example 3

Solubilities

The solubility of the crystalline calcium salt of 5-methyl-(6S)-tetrahydrofolic acid is given in the following Table:

Type	Solubility at 20° C. in	
	0.9% NaCl	water
Type I	1.6%	1.1%
Type II	5.8%	3.8%
Type III	1.5%	1.0%

Example 4

Amorphous Calcium Salt of 5-Methyl-(6S)-tetrahydrofolic Acid

7.5 g 5-methyl-(6S)-tetrahydrofolic acid were introduced into 75 ml water at room temperature whilst passing N<sub>2</sub> into

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the batch, and the batch was adjusted to pH 12 with aqueous 30% sodium hydroxide solution. The clear solution which was thus obtained was adjusted to pH 7.5 with 37% hydrochloric acid and was treated with a solution of 7.15 g calcium chloride 6H<sub>2</sub>O in 11.7 ml water. The white suspension which was formed was stirred for 5 hours and was then filtered under suction at room temperature. The solid was washed with water and was dried under vacuum at 45° C.

5.8 g of a white, amorphous calcium salt of 5-methyl-(6S)-tetrahydrofolic acid were obtained, which had a content of 98.0% and a 6S fraction corresponding to 99.6%.

Even after treating this substance at 60° C. in a humidity cabinet, no crystalline fractions could be determined either under a polarising microscope or by X-ray diffraction measurements.

## Example 5

## Crystalline Calcium Salt of 5-Methyl-(6 R,S)-tetrahydrofolic Acid

70 g 5-methyl-(6R, S)-tetrahydrofolic acid were placed in a vessel in 780 ml water and the batch was adjusted to pH 7.5 with 45.2 g of 30% NaOH. The clear, slightly reddish solution was treated with a solution of 62.7 g calcium chloride 6H<sub>2</sub>O in 140 ml water, and the solid was filtered off and washed with a little water. The crude product which was thus obtained was suspended in water and treated at 90° C. for 24 hours.

74.0 g of a white, crystalline calcium salt of 5-methyl-(6R,S)-tetrahydrofolic acid was obtained, with a content of 99.1%.

## Example 6

## Crystalline Calcium Salt of 5-Methyl-(6R)-tetrahydrofolic Acid

16.5 g 5-methyl-(6R)-tetrahydrofolic acid were placed in a vessel in 100 ml water at 92° C. with 50 g calcium chloride 6H<sub>2</sub>O. The clear, slightly yellowish suspension was stirred for 10 minutes at 91° C., and the solid was filtered off, washed with a little water and dried at 35° C. under vacuum.

15.4 g of a light beige crystalline calcium salt of 5-methyl-(6R)-tetrahydrofolic acid were obtained, with a content of 97.9% and a water content of 7.8%.

## Example 7

## Type I

130 kg water were placed in a vessel and 12.8 kg 5-methyl-(6S)-tetrahydrofolic acid were introduced. The pH was adjusted to 11.6 with about 9.1 kg of 30% NaOH, and was then adjusted to 7.6 with about 1.9 kg of 37% hydrochloric acid. A suspension containing 0.3 kg carbon and 0.3 kg Cellflock was added to the clear solution. The solid was filtered off and washed with 13 litres of water. The filtrate was treated with a solution containing 8.3 kg calcium chloride 2H<sub>2</sub>O, heated to 90° C. and stirred for 30 minutes. The product was filtered hot and was washed with 2×20 kg water. The moist crude product which was thus obtained was slurried in 115 litres of water, heated to 90° C., immediately filtered hot, washed with 2×20 kg water, and dried at 40° C. under vacuum.

11.6 kg of a white, crystalline calcium salt of 5-methyl-(6S)-tetrahydrofolic acid (Type I) were obtained, which had a purity of 99.0% and a water content of 14.5%.

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## Example 8

## Type I

1600 ml water were placed in a vessel and 194 g 5-methyl-(6S)-tetrahydrofolic acid were introduced. The pH was adjusted to 7.0 with about 80 ml of 30% NaOH. A suspension containing 20 g carbon and 20 g Cellflock in 190 ml water was added to the clear solution. The solid was filtered off and washed with water. The filtrate was treated with 950 ml of a 5.5 M calcium chloride solution, heated to 90° C. and stirred for 60 minutes. The product was filtered hot, washed with water, and dried at 45° C. under vacuum.

156.2 g of a white, crystalline calcium salt of 5-methyl-(6S)-tetrahydrofolic acid (Type I) were obtained, with a purity of 99.7% and a 6S fraction of 99.9%.

## Example 9

## Type 1 and Conversion Into Type II

554 g water were placed in a vessel and 53.1 g 5-methyl-(6S)-tetrahydrofolic acid were introduced. The pH was adjusted to 7.5 with 30% NaOH. 1.3 g carbon, 1.3 g Cellflock and 19.5 g water were added to the clear solution. The suspension was filtered and the solid was washed with 55 ml water. The filtrate was treated with a solution of 52.0 g calcium chloride 6H<sub>2</sub>O in 84.6 g water, and was heated to 90° C. and seeded with 100 mg of the crystalline calcium salt of 5-methyltetrahydrofolic acid. After crystallisation had occurred, the product was filtered hot at 90° C. and was washed with 2×103 g water. The moist crude product which was thus obtained was slurried in 480 ml water, heated to 90° C., immediately filtered hot, washed as above, and dried at 45° C. under vacuum.

47.5 g of a white, crystalline calcium salt of 5-methyl-(6S)-tetrahydrofolic acid (Type I) were obtained, with a purity of 98.8% and a water content of 12.2%.

This Type I modification could be converted into the Type II modification with a water content of 5.0% by drying it at 70° C. under vacuum for 30 minutes.

## Example 10

## Type III

15.8 g of the calcium salt of 5-methyl-(6S)-tetrahydrofolic acid were heated to 95° C. in 140 ml water whilst passing N<sub>2</sub> through the batch. After 30 minutes at 95° C. the white suspension was filtered hot under suction, and the solid was washed with water and dried at 35° C. under vacuum.

14.0 g of a white, crystalline calcium salt of 5-methyl-(6S)-tetrahydrofolic acid (Type III) was obtained, with a content of 98.9% and a 6S fraction of 99.9%.

## Example 11

## Type IV

20.0 g of the calcium salt of 5-methyl-(6S)-tetrahydrofolic acid were heated to 100° C. in 180 ml water whilst passing N<sub>2</sub> through the batch. After 30 minutes at 100° C. the white suspension was filtered hot under suction, and the solid was washed with water and dried at 25° C. under vacuum.

16.9 g of a white, crystalline calcium salt of 5-methyl-(6S)-tetrahydrofolic acid (Type IV) were obtained, with a content of 98.3% and a water content of 9.9%.

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By drying it at 65° C. under vacuum, the water content of this product could be reduced to 5.5% without a different crystalline modification being obtained in the course of this procedure.

### Examples of Uses of the Crystalline Material

(a) As constituent for the production of drugs:

Beside the well known uses of 5-formyltetrahydrofolic acid previously described, reference is invited to U.S. Ser. No. 09/095,572 for pharmaceutical preparations including pharmaceutically acceptable excipients, as well as dosage ranges of 5-methyltetrahydrofolic acid.

(b) As food additive:

The range of possible applications as food additive is very wide (from single preparations to multivitamin preparations and grain to breakfast foods). Please refer to e.g. WO 97/27764, SAMSF/Bailey et al., for examples (e.g. examples 1, 2, 6, 8 and 9 all contain formulas with 5-methyltetrahydrofolic acid).

(c) For the production of other tetrahydrofolic acid derivatives:

Under "other tetrahydrofolic acid derivatives", other salts of 5-methyltetrahydrofolic acid and the free acid itself are especially included.

The preceding examples can be repeated with similar success by substituting the generically or specifically described reactants and/or operating conditions of this invention for those used in the preceding examples. Also, the preceding specific embodiments are to be construed as merely illustrative, and not limitative of the remainder of the disclosure in any way whatsoever.

The entire disclosure of all applications, patents and publications, cited above and below, and of corresponding Swiss application 695/99, are hereby incorporated by reference.

From the foregoing description, one skilled in the art can easily ascertain the essential characteristics of this invention, and without departing from the spirit and scope thereof, can make various changes and modifications of the invention to adapt it to various usages and conditions.

TABLE 1

Crystalline calcium salt of 5-methyl-(6S)-tetrahydrofolic acid (Type I)

Diffractionmeter : Transmission  
Monochromator : Curved Ge (111)  
Wavelength : 1.540598 Cu  
Detector : Linear PSD  
Scan Mode : Debye-Scherrer/Moving PSD/Fixed omega  
2Theta scan

! Peak search parameters : Expected halfwidth : .150  
! Significance level : 2.5  
! Peak height level : 10

Peaklist [Range 1 : 2Theta = 5.000 34.980 .020 Imax = 765]

!	D	2Theta	I (rel)	I (abs)	FWHM	h	k	l
	13.474630	6.5544	100.0	755	.2200			
	8.979750	9.8420	18.5	140	.1600			
	6.936035	12.7526	20.3	153	.1600			
	6.662427	13.2786	38.3	289	.0800			
	6.497896	13.6164	29.4	222	.1200			
	6.323596	13.9935	18.8	142	.0200			
	6.148863	14.3933	14.0	106	.0400			
	5.966675	14.8352	15.5	117	.1200			
	5.593548	15.8309	27.5	208	.2200			
	5.368022	16.5006	19.7	149	.1127			
	5.282104	16.7709	42.5	321	.2000			
	4.977751	17.8044	23.6	178	.1800			
	4.672452	18.9782	32.7	247	.2800			
	4.411916	20.1102	34.8	263	.0800			

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TABLE 1-continued

Crystalline calcium salt of 5-methyl-(6S)-tetrahydrofolic acid (Type I)

5    Diffractometer        : Transmission  
Monochromator        : Curved Ge (111)  
Wavelength            : 1.540598 Cu  
Detector                : Linear PSD  
Scan Mode             : Debye-Scherrer/Moving PSD/Fixed omega  
2Theta scan

10 ! Peak search parameters : Expected halfwidth : .150  
!                                Significance level : 2.5  
!                                Peak height level : 10

Peaklist [Range 1 : 2Theta = 5.000 34.980 .020 Imax = 765]

!	D	2Theta	I (rel)	I (abs)	FWHM	h	k	l
15	4.257688	20.8467	34.2	258	.2600			
	3.761157	23.6360	13.3	100	.0400			
	3.699455	24.0361	22.3	168	.1400			
	3.558431	25.0037	14.8	112	.1000			
	3.439070	25.8864	21.0	159	.1400			
20	3.272550	27.2283	22.1	167	.2800			
	3.218939	27.6907	17.0	129	.1400			
	3.140884	28.3931	17.2	130	.0800			
	3.013536	29.6198	13.9	105	.1000			
	2.873482	31.0991	15.1	114	.0200			
	2.782802	32.1395	16.6	125	.0200			
25	2.754830	32.4748	20.2	152	.0600			
	2.713309	32.9858	15.4	116	.1127			

TABLE 2

30 Crystalline calcium salt of 5-methyl-(6S)-tetrahydrofolic acid (Type II)

Diffractionmeter : Transmission  
Monochromator : Curved Ge (111)  
Wavelength : 1.540598 Cu

35 Detector : Linear PSD  
Scan Mode : Debye-Scherrer/Moving PSD/Fixed omega  
2Theta scan

! Peak search parameters : Expected halfwidth : .150  
! Significance level : 2.5  
! Peak height level : 10

40 Peaklist [Range 1 : 2Theta = 5.000 34.980 .020 Imax = 526]

!	D	2Theta	I (rel)	I (abs)	FWHM	h	k	l
	12.720530	6.9434	100.0	517	.2600			
	8.508053	10.3891	29.4	152	.2400			
	6.631466	13.3409	19.6	101	.1200			
45	5.883504	15.0461	71.2	368	.2200			
	5.580025	15.8696	27.8	144	.0800			
	5.010988	17.6854	42.5	220	.1400			
	4.730443	18.7434	53.6	277	.1400			
	4.215807	21.0561	35.5	184	.0400			
	3.943879	22.5263	38.8	201	.3600			
50	3.581969	24.8368	24.8	128	.0200			
	3.493985	25.4726	29.6	153	.0400			
	3.309171	26.9212	22.7	117	.0200			

TABLE 3

Crystalline calcium salt of  
5-methyl-(6S)-tetrahydrofolic acid (Type III)

Diffractometer : Transmission  
60 Monochromator : Curved Ge (111)  
Wavelength : 1.540598 Cu  
Detector : Linear PSD  
Scan Mode : Debye-Scherrer/Moving PSD/Fixed omega  
2Theta scan  
! Peak search parameters : Expected halfwidth : .150  
! Significance level : 2.5  
65 ! Peak height level : 10  
Peaklist [Range 1 : 2Theta = 5.000 34.980 .020 Imax = 817]

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!	D	2Theta	I (rel)	I (abs)	FWHM	h	k	l
	12.933490	6.8289	100.0	786	.1200			
	11.036740	8.0043	18.9	149	.0400			
	9.945525	8.8842	18.4	145	.1000			
	8.877709	9.9554	12.4	98	.0796			
	8.640580	10.2293	49.6	390	.1000			
	7.873330	11.2292	6.4	50	.1000			
	7.144004	12.3799	7.6	59	.0800			
	6.948557	12.7295	20.3	159	.1000			
	6.659956	13.2835	10.1	80	.0400			
	6.466239	13.6834	7.6	60	.0200			
	6.305060	14.0349	37.6	296	.1000			
	6.154434	14.3802	16.4	129	.0400			
	6.057193	14.6123	15.3	121	.0600			
	5.920458	14.9517	17.6	139	.1000			
	5.738533	15.4285	48.9	385	.1000			
	5.530167	16.0136	30.3	238	.1000			
	5.322477	16.6428	18.1	143	.0600			
	5.245302	16.8894	47.4	372	.0800			
	5.154604	17.1888	20.9	164	.0796			
	5.038273	17.5888	30.8	242	.1000			
	4.980502	17.7945	10.7	84	.0796			
	4.759336	18.6286	31.6	248	.1200			
	4.702846	18.8544	24.3	191	.0796			
	4.575841	19.3827	15.6	122	.0800			
	4.478961	19.8061	25.9	204	.1000			
	4.377158	20.2716	48.1	378	.1000			
	4.309006	20.5957	11.9	93	.0796			
	4.242777	20.9207	31.3	246	.0800			
	4.051441	21.9207	10.3	81	.0200			
	3.940356	22.5467	67.8	533	.1200			
	3.782452	23.5010	12.4	98	.0400			
	3.609291	24.6458	9.5	75	.0200			
	3.523157	25.2582	27.0	212	.2000			
	3.460874	25.7205	43.4	341	.0800			
	3.408545	26.1223	12.4	98	.0796			
	3.341048	26.6596	16.1	127	.2000			
	3.273575	27.2196	28.4	223	.1400			
	3.188038	27.9645	12.6	99	.0200			
	3.160110	28.2168	12.5	98	.0400			
	3.103472	28.7427	15.0	118	.0800			
	3.052658	29.2317	13.9	109	.0600			
	3.017419	29.5808	27.7	218	.1400			
	2.970195	30.0621	10.6	83	.1200			
	2.921067	30.5800	13.9	109	.0200			
	2.899222	30.8161	9.6	76	.0796			
	2.870572	31.1314	9.6	75	.0400			
	2.830661	31.5817	11.0	86	.0200			
	2.758126	32.4349	11.3	89	.0400			
	2.733265	32.7382	13.2	104	.0600			
	2.695836	33.2058	13.7	108	.0800			
	2.660160	33.6643	11.7	92	.1000			
	2.609572	34.3369	9.2	72	.0200			

TABLE 4

Crystalline calcium salt of 5-methyl-(6S)-tetrahydrofolic acid (Type IV)								
Diffractometer	: Transmission							
Monochromator	: Curved Ge (111)							
Wavelength	: 1.540598 Cu							
Detector	: Linear PSD							
Scan Mode	: Debye-Scherrer/Moving PSD/Fixed omega							
2Theta scan								
! Peak search parameters	: Expected halfwidth : .150							
! Significance level	: 2.5							
! Peak height level	: 10							
Peaklist [Range 1 : 2Theta = 5.000 34.980 .020 I <sub>max</sub> = 473]								
!	D	2Theta	I (rel)	I (abs)	FWHM	h	k	l
	13.398610	6.5916	97.7	446	.1600			
	12.930100	6.8307	100.0	457	.0915			
	11.033220	8.0069	19.2	88	.0800			
	9.952926	8.8776	16.7	76	.1200			
	8.912272	9.9167	25.5	116	.1600			

TABLE 4-continued

Crystalline calcium salt of 5-methyl-(6S)-tetrahydrofolic acid (Type IV)								
Diffractometer	: Transmission							
Monochromator	: Curved Ge (111)							
Wavelength	: 1.540598 Cu							
Detector	: Linear PSD							
Scan Mode	: Debye-Scherrer/Moving PSD/Fixed omega							
10 2Theta scan								
! Peak search parameters	: Expected halfwidth : .150							
! Significance level	: 2.5							
! Peak height level	: 10							
Peaklist [Range 1 : 2Theta = 5.000 34.980 .020 I <sub>max</sub> = 473]								
15 !	D	2Theta	I (rel)	I (abs)	FWHM	h	k	l
	8.626970	10.2455	48.9	223	.0800			
	6.931997	12.7600	37.4	171	.1000			
	6.651761	13.3000	39.7	181	.1200			
	6.499623	13.6127	32.8	150	.0800			
	6.309299	14.0254	47.0	215	.1600			
20	6.161306	14.3641	25.1	115	.1200			
	5.917463	14.9593	27.0	124	.1000			
	5.736254	15.4347	49.8	227	.0800			
	5.544314	15.9724	36.7	168	.1600			
	5.255854	16.8553	62.1	284	.2400			
	5.172075	17.1303	29.5	135	.0915			
25	5.035719	17.5978	37.0	169	.1200			
	4.978813	17.8006	31.3	143	.0400			
	4.758441	18.6321	40.7	186	.1000			
	4.688853	18.9112	46.0	210	.0915			
	4.577465	19.3757	29.5	135	.0915			
	4.479376	19.8043	35.5	162	.1000			
30	4.383704	20.2410	63.6	290	.1200			
	4.246196	20.9037	59.5	272	.1400			
	4.088125	21.7216	19.7	90	.0200			
	3.941748	22.5386	62.9	288	.1400			
	3.778991	23.5229	27.9	128	.0400			
	3.696576	24.0551	30.5	139	.1000			
35	3.523769	25.2537	35.6	163	.2400			
	3.459683	25.7295	44.7	204	.0800			
	3.338511	26.6803	28.7	131	.0200			
	3.273450	27.2206	45.5	208	.1000			
	3.135320	28.4446	23.6	108	.0600			
	3.108154	28.6985	25.9	118	.0200			
40	3.018687	29.5681	34.4	157	.1400			
	2.923031	30.5589	21.9	100	.0200			
	2.844431	31.4249	18.4	84	.0200			
	2.749393	32.5408	28.5	130	.1200			
	2.713739	32.9804	25.6	117	.0200			
	2.663207	33.6246	19.6	90	.0600			
45	2.613490	34.2838	17.4	80	.0200			

What is claimed is:

1. A crystalline salt of 5-methyl-(6R,S)-, -(6S)- or -(6R)-tetrahydrofolic acid said crystalline salt having a water of crystallization of at least one equivalent per equivalent of 5-methyltetrahydrofolic acid.
2. A crystalline salt according to claim 1, of 5-methyl-(6S)- or -(6R)-tetrahydrofolic acid.
3. A crystalline calcium salt according to claim 1, of 5-methyl-(6S)- and -(6R)-tetrahydrofolic acid having  $\geq 3$  equivalents of water.
4. A crystalline calcium salt of 5-methyl-(6S)-tetrahydrofolic acid with 2 theta values of 6.5, 13.3, 16.8 and 20.1 (Type I) said crystalline salt having a water of crystallization of at least one equivalent per equivalent of 5-methyltetrahydrofolic acid.
5. A crystalline calcium salt according to claim 1, of 5-methyl-(6S)-tetrahydrofolic acid with 2 theta values of 5.3, 6.9, 18.7 and 21.1 (Type II).
6. A crystalline calcium salt according to claim 1, of 5-methyl-(6S)-tetrahydrofolic acid with 2 theta values of 6.8, 10.2, 15.4 and 22.5 (Type III).

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7. A crystalline calcium salt according to claim 1, of 5-methyl-(6S)-tetrahydrofolic acid with 2 theta values of 6.6, 15.9, 20.2 and 22.5 (Type IV).

8. A method of producing crystalline salts of 5-methyl-(6R,S)-, -(6S)- and 5-methyl-(6R)-tetrahydrofolic acid, comprising subjecting a salt of 5-methyl-(6R,S)-, -(6S)- or -(6R)-tetrahydrofolic acid in a polar medium to a thermal treatment, at a temperature above 60° C., and thereafter crystallizing said salt from the resultant heated solution.

9. A method according to claim 8, wherein the crystallisation is effected after thermal treatment at a temperature above 85° C.

10. A method according to claim 8, wherein the crystallisation is effected from a solution.

11. A method according to claim 8, wherein the crystallisation is effected from a suspension.

12. A method according to claim 10, characterised in that crystallisation is effected from water or from a mixture of water and an organic solvent which is miscible with water.

13. A method according to claim 8, wherein said salt is an alkaline earth salt.

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14. A method according to claim 8, wherein said salt is calcium.

15. A method of producing 5-methyl-(6S)-tetrahydrofolic acids with 2 theta values of 5.3, 6.9, 18.7 and 21.1 (Type II) comprising drying sufficiently 5-methyl-(6S)-tetrahydrofolic acid with 2 theta values of 6.5, 13.3, 16.8 and 20.1 (Type I).

16. A method of producing 5-methyl-(6S)-tetrahydrofolic acid with 2 theta values of 6.8, 10.2, 15.4 and 22.5 (Type III) comprising subjecting to sufficient thermal treatment at above 90° C., a crystalline calcium salt of 5-methyl-(6S)-tetrahydrofolic acid with 2 theta values of 6.5, 13.3, 16.8 and 20.1 (Type I).

17. A method of producing 5-methyl-(6S)-tetrahydrofolic acid with 2 theta values of 6.6, 15.9, 20.2, 22.5 (Type IV) comprising subjecting to sufficient thermal treatment at above 95 ° C., a crystalline calcium salt of 5-methyl-(6S)-tetrahydrofolic acid with 2 theta values of 6.5, 13.3, 16.8 and 20.1 (Type I).

\* \* \* \* \*

UNITED STATES PATENT AND TRADEMARK OFFICE  
**CERTIFICATE OF CORRECTION**

PATENT NO. : 6,441,168 B1  
DATED : August 27, 2002  
INVENTOR(S) : Rudolf Muller et al.

Page 1 of 1

It is certified that error appears in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

Column 12,

Line 4, reads "acids," should read -- acid --

Signed and Sealed this

First Day of June, 2004

A handwritten signature in black ink, reading "Jon W. Dudas". The signature is stylized, with a large, looped initial "J" and a cursive "Dudas".

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JON W. DUDAS  
*Acting Director of the United States Patent and Trademark Office*